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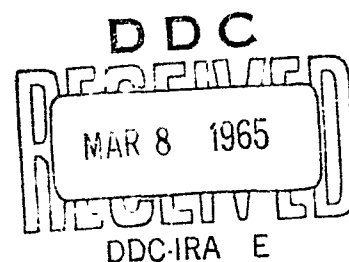
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TECHNICAL MANUSCRIPT 202

GREENHOUSE TESTS TO COMPARE
EUROPEAN AND BELTSVILLE ISOLATES
OF PERONOSPORA TABACINA

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GREENHOUSE TESTS TO COMPARE EUROPEAN AND
BELTSVILLE ISOLATES OF PERONOSPORA TABACINA

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ABSTRACT.

Of the tobaccos used to differentiate a European and the Beltsville isolate of Peronospora tabacina, sporulation occurred on Nicotiana goodspeedii with the European but not with the Beltsville isolate. This situation was reversed on Bel 62-502-1 (N. tabacum selection). Water-soluble germination inhibitors are present in both fungus isolates. Germination of the two isolates in response to temperature is similar. The European may sporulate more profusely on a susceptible host than does the Beltsville isolate; it also has a narrower temperature range for sporulation.

I. INTRODUCTION

A study was designed to determine the differences between a European isolate of Peronospora tabacina and an endemic isolate of this organism used in the USDA laboratories at Beltsville. Dr. Paul R. Miller, USDA, collected the European isolate in the vicinity of Stuttgart, Germany, in the summer of 1963. Dr. Howard E. Heggstad, USDA, supplied seed of tobacco species and varieties as well as the Beltsville isolate of the fungus. Precautions were taken to prevent the escape of the European isolate by growing it inside a cubicle in a greenhouse equipped with filters to remove spores from incoming and outgoing air.¹

II. MATERIALS AND METHODS

Plants were grown from seed in 3-inch clay pots in a soil mixture of 1/3 peat moss and 2/3 sandy loam. Miller's VHPF fertilizer was used as a soil drench according to directions on the package. Plants were grown in a growth room under a combination of fluorescent and incandescent lights at about 800 foot candles during a 12-hour day. Temperature cycled between 65 F at night and a maximum of 75 F during the day.

Inoculations were made on 5- to 12-week-old plants with spores formed within 48 hours of inoculation. Spores in water were atomized upon plants in dew cabinets² at 65 F. After 48 hr in dew, plants were returned to the cubicle. Ten days after inoculation plants were placed in dew overnight, then returned to the cubicle for observations.

For the spore germination studies, spore suspensions were prepared in sterile distilled water and diluted in a series of water blanks. Spore concentrations were standardized by atomizing spores onto 2% Difco water agar or on sterile glass slides from a known distance for a known time. After incubation in the dark at selected temperatures for given periods, cultures were stained with 1% cotton blue-lactophenol-acid fuchsin. Three fields of 100 spores each were observed for each replication.

III. RESULTS

Responses of several Nicotiana species and tobacco selections to infection by both isolates are found in Table 1.

Young seedlings of susceptible varieties do not survive infection by either isolate when the inoculum dosage is high. The young plants display a severe wilt as part of the dying syndrome. Even though older plants of certain varieties showed some wilting, they sustained most injury by loss of chlorophyll and by necrosis. However, the magnitude of sporulation on those varieties responding with extensive necrotic lesions was less than on varieties with chlorotic lesions. The Beltsville isolate induced greater necrosis, consequently, a lesser degree of sporulation. Quantitative measurements of sporulation were not made. Sporulation occurred only once on infected tissue, even though the area remained green and in apparently active physiological condition. Acquired resistance and immunity have been observed by Clayton et al.,³ Cruickshank and Mandryk,⁴ and Mandryk,⁵ who reported a systemic type of resistance after initial infection. Both isolates infected three-month-old plants.

Bacto Difco agar was a better substrate for germination than glass, as has been shown by Shepherd⁶ (Fig. 1). Counts made within 12 hours of incubation at 65 F are more accurate than later counts because of intertwining of germ tubes.

Drop-off in germination with increasing spore concentration shown in Figures 2 and 3 can be explained by the presence of germination inhibitors⁷ produced by both isolates. A 2% water agar negated some of the inhibition. It is assumed that the agar colloid adsorbs some of the inhibitor.

Germination of the two isolates in response to temperature follows the same trend (Fig. 4). Optimum germination after 12 hours occurs between 55 and 65 F. This is in agreement with reports in the literature.

Sporulation occurred 7 days after incubation of leaf discs cut from inoculated leaves (Table 2). The discs were floated on water in petri plates and incubated at temperatures between 50 and 86 F. (The Beltsville isolate sporulated at 86 F; the European isolate did not). Sporulation was assessed by agitating discs in 8 ml of water and examining the suspension microscopically. Since not all areas of a leaf are uniformly infected, it is possible that some discs might have been removed from areas where little infection was present.

TABLE 1. RESPONSE OF SELECTED VARIETIES OF TOBACCOS TO INOCULATION
WITH EUROPEAN AND BELTSVILLE ISOLATES OF P. TABACINA

Tobacco selection	European Isolate				Beltsville Isolate			
	Distor- tion	Chlor- osis	Necro- sis	Sporu- lation	Distor- tion	Chlor- osis	Necro- sis	Sporu- lation
Ca 704-2	+	+	+	trace	+	+	+	trace
Bel 62-14-7	+	+	+	trace	+	+	+	trace
Bel 62-517-1	+	+	+	-	+	+	+	-
Bel 61-12	+	+	+	-	+	+	+	-
<u>Nicotiana debneyi</u> ^{a/}	+	+	+	-	+	+	+	-
<u>Samsoun</u> ^{b/}	-	++	++	+++	-	+	+	++
Virginia Gold ^{b/}	-	++	++	+++	-	+	+	++
<u>N. goodspeedii</u> ^{c/}	+	+	+	+	+	+	+	-
Bel 62-502-1	+	+	+	-	+	+	+	+

a. Leaves pitted with both European and Beltsville isolates.

b. Wilting and death more frequent and more rapid with European isolate.

c. Numerous necrotic stem lesions with both isolates.

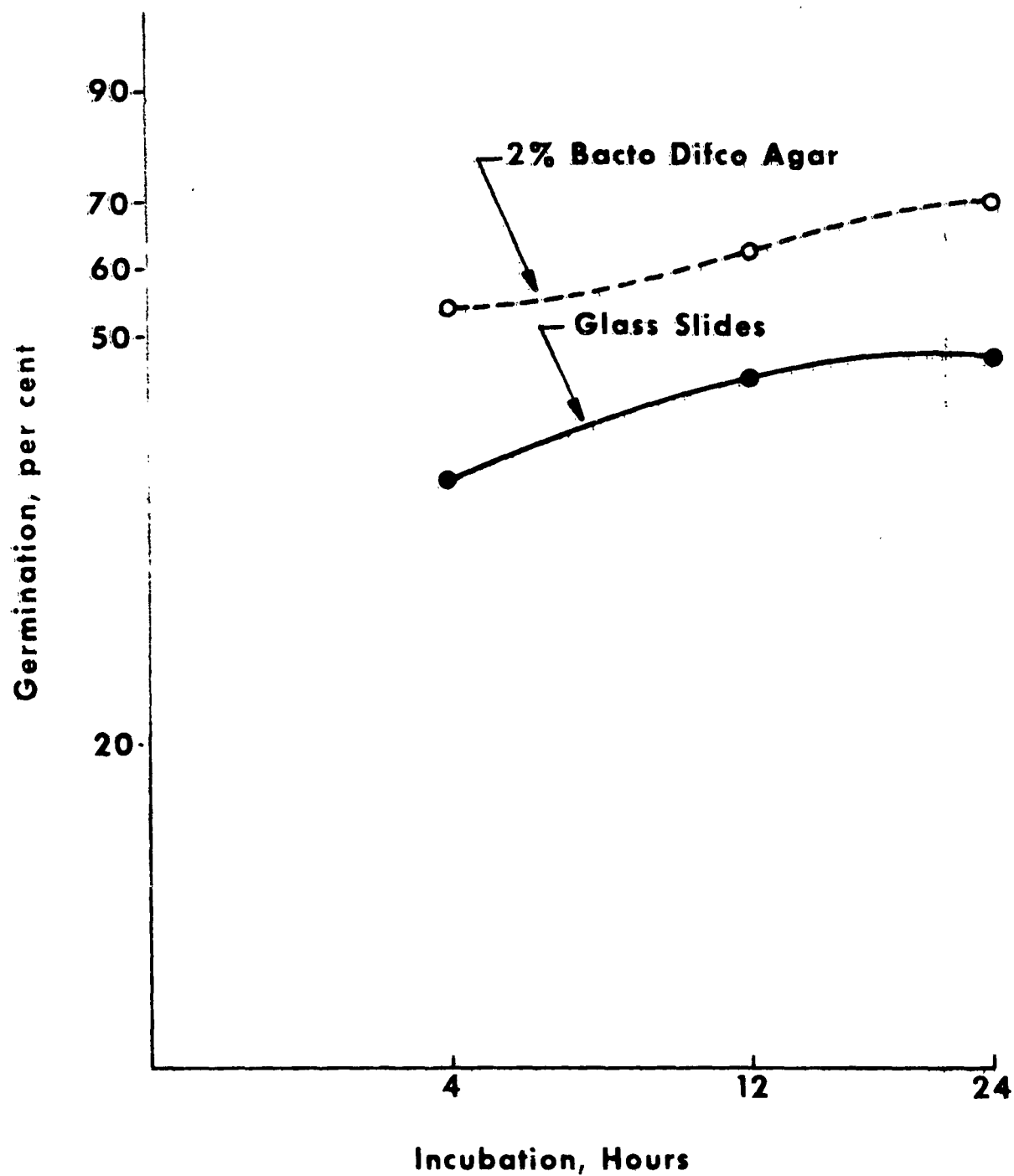


Figure 1. Germination of *P. tabacina* conidia as Affected by Time. Incubation temperature - 65 F. Each point is the mean of 1800 observations.

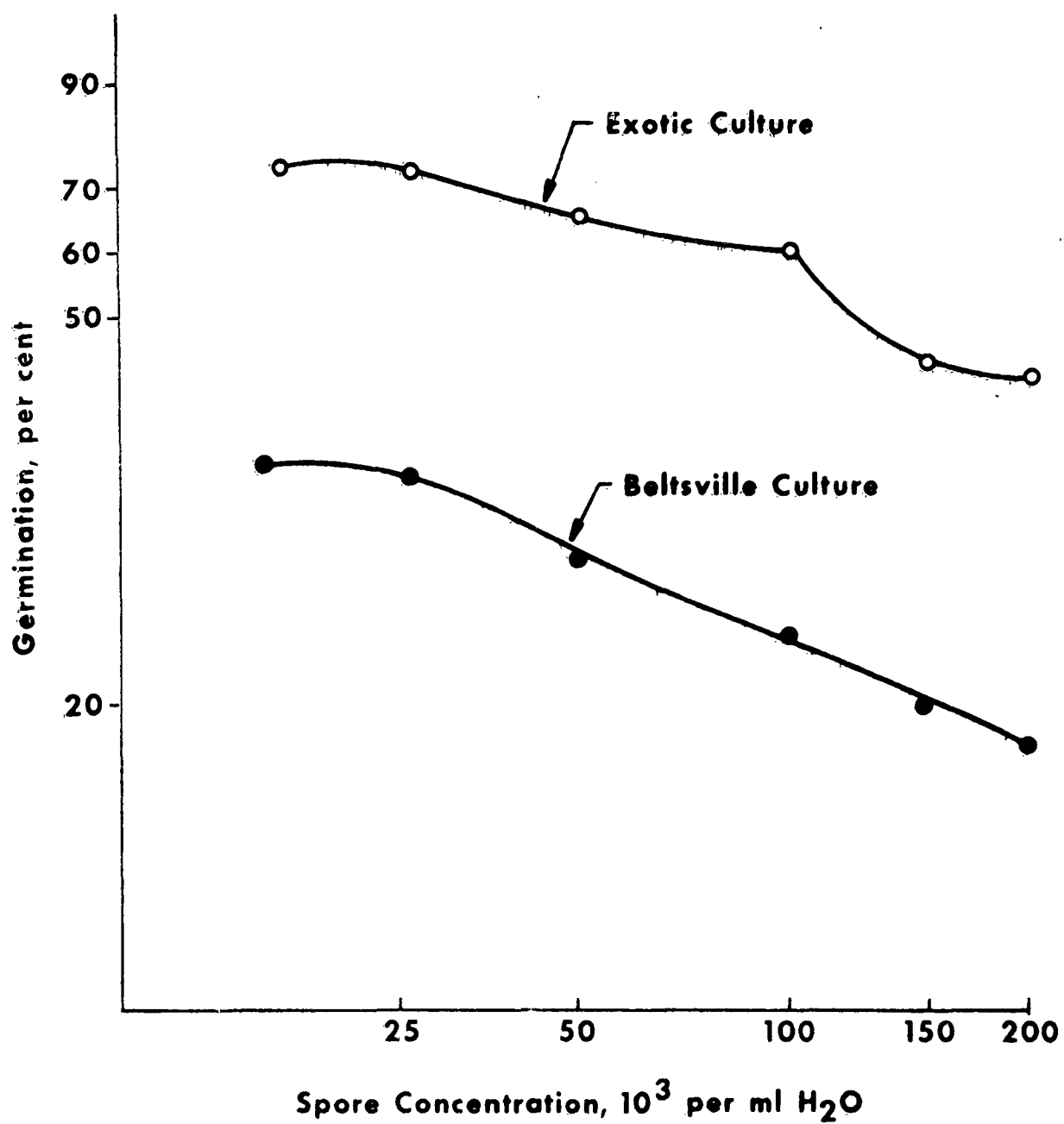


Figure 2. Effect of Spore Concentration on Germination of *P. tabacina* conidia Incubated at 65 F on 2% Difco Agar. Each point represents the mean of 1200 observations.

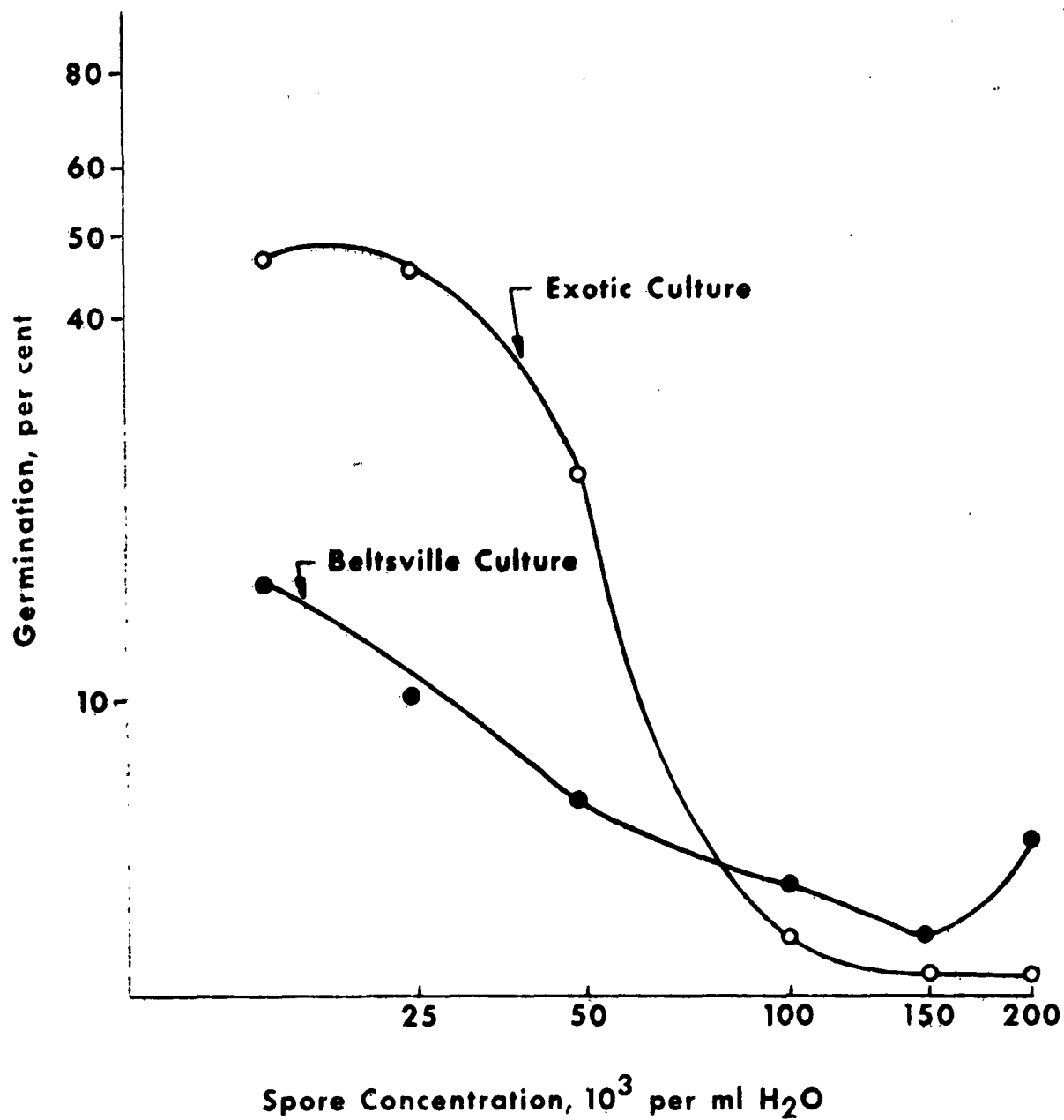


Figure 3. Effect of Spore Concentration on Germination of *P. tabacina* conidia Incubated at 65 F on Sterile Glass Slides.

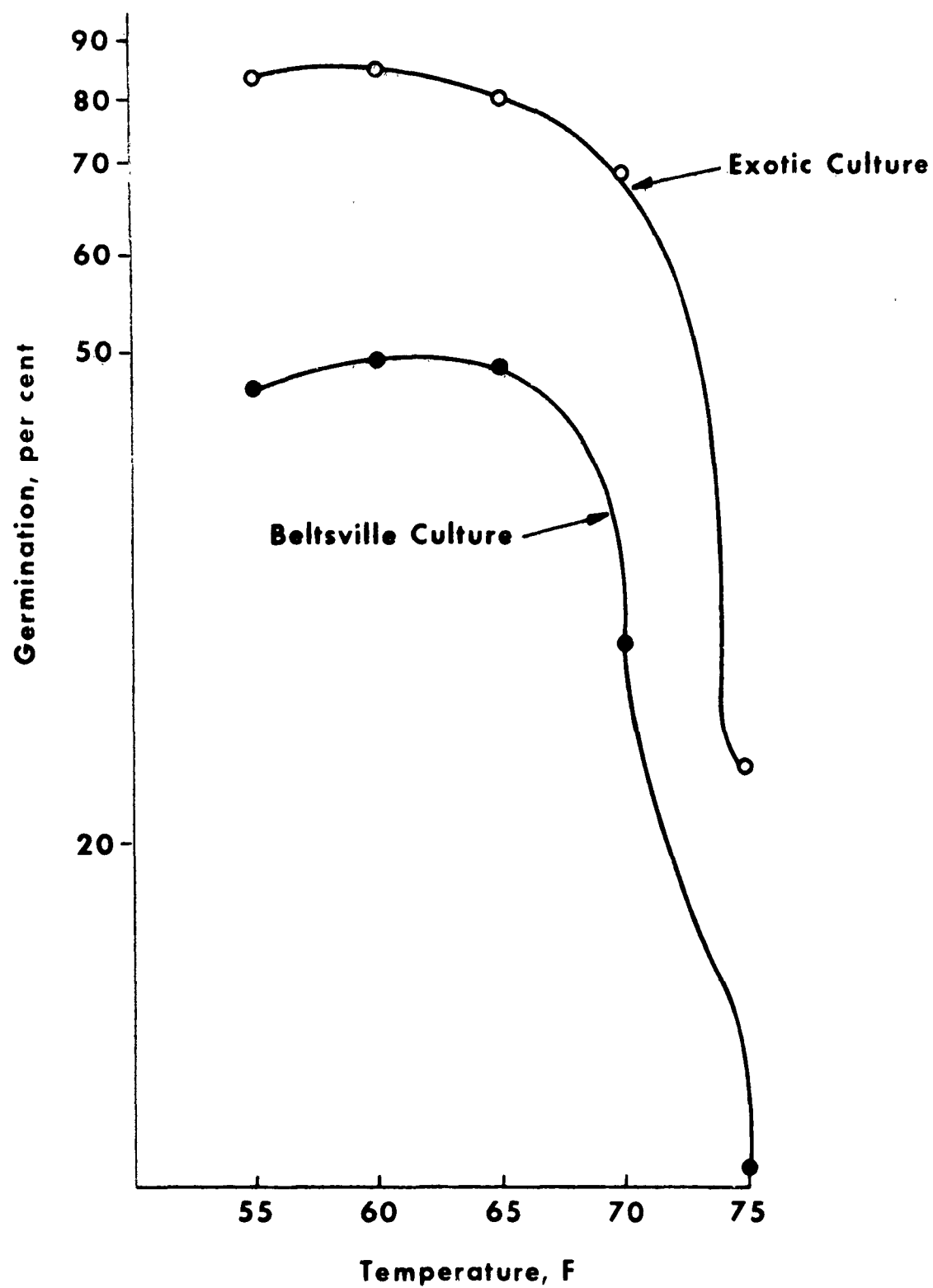


Figure 4. Effect of Temperature on Germination of *P. tabacina* conidia after Eight Hours' Incubation on 2% Bacto Difco Agar.

TABLE 2. SPORULATION OF BELTSVILLE AND EUROPEAN ISOLATES OF
PERONOSPORA TABACINA ON LEAF DISCS ON WATER AT 5
TEMPERATURES

Replicate	Temperature, F				
	50	59	68	77	86
Beltsville Isolate					
1	+ ^{a/}	-	+	+	+
2	+	+	+	-	+
3	+	+	-	+	+
4	+	+	+	+	+
5	+	+	+	+	-
6	+	+	-	+	-
European Isolate					
1	+	+	+	-	-
2	+	+	+	-	-
3	+	+	+	-	-
4	+	+	-	-	-
5	+	+	+	+	-
6	+	+	+	-	-

a. + = sporulation; - = no sporulation.

IV. DISCUSSION AND CONCLUSIONS

The two isolates of P. tabacina appear to be different strains. The virulence of the two cultures has not been ascertained, but certain differences between them are now apparent. The European isolate seems to have the ability to sporulate more profusely on a susceptible host, to cause death by wilting to a greater extent, and to have a more narrow range of sporulation temperature. Both isolates are capable of causing seed bed and field infections, of infecting all the varieties tested, and of sporulating only once in a given area of infected tissue. Unfavorable environmental conditions in fields in this country appear to be the reason that local strains are not very destructive on field plants but cause most damage in seed beds.

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